Analysis of mitotic and meiotic chromosomes of the European hamster, *Cricetus cricetus* (L.)

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Introduction

The various staining techniques recently developed (e. g. Caspersson et al. 1970, Arrighi and Hsu 1971, Schnedl 1971, Sumner et al. 1971, Müller and Rosenkranz 1972, Seabright 1972, Sumner 1972) simplify karyological analyses of mammalian chromosomes. The application of G-banding technique allows the identification of each chromosome of a complement, C-banding exhibits the distribution of heterochromatin.

Several hamster species *(Cricetulus griseus, Cricetulus barabensis, Cricetulus migratorius, Cricetus cricetus* etc.) are favourable animals for cytogenetical studies because of their low number of chromosomes mainly meta- and submetacentric.

For our investigations, we have chosen *Cricetus cricetus*, a species with a chromosome number of $2n = 22$. We intend to give a representation of G-bands and C-bands in mitotic chromosomes and, in addition, C-bands in meiotic chromosomes.

Materials and methods

For this study, we used four male and four female European hamsters captured in the surroundings of the "Neusiedler See" (Eastern Austria).

Chromosome preparations were obtained from fibroblast cultures. After colcemid-treatment and hypotonizing with KCl-solution, the cells were fixed with acetic-methanol (1:3) and air-dried. Two different procedures were used for G-banding: ASG-technique (Sumner et al. 1971) and pancreatin treatment (Müller and Rosenkranz 1972, modified). The distribution of heterochromatin in mitotic and meiotic chromosomes was shown by a modified C-staining technique of Sumner (1972).

Meiotic preparation was done by the air-drying method of Evans et al. (1964).

In order to determine length and arm ratio of the chromosomes, we used the LEITZ-"Classimat" (Rosenkranz 1973).

Results

I. Mitotic chromosomes

The normal karyotype of *Cricetus cricetus* consists of 18 metacentric and submetacentric and 2 acrocentric autosomes plus 2 sex chromosomes. Most of them are easily distinguishable from one another on basis of length and arm ratio.

We arranged the chromosomes according to the proposal of Fredga and Santesson (1964). There are two reasons why we did not accept the arrangement of Hsu and Benirschke (1970):

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1. In many cases the submetacentric chromosome which Hsu and Benirschke place in third position is longer than that placed in second position.
2. When the submetacentric chromosome is placed as no. 5, the similarity to the karyotype of the Chinese hamster becomes obvious.

We have measured the length and calculated the arm ratio of the chromosomes in 20 mitoses; the data for each chromosome are averaged and presented in the table. The table supports the statement previously made, that chromosome no. 5 is often longer than no. 2. In addition to this, data for the chromosomes nos. 8 and 9 show that the difference in length is very slight and that — in some cases — chromosome no. 9 is longer than no. 8.

G-bands: G-banding patterns, which allow the unequivocal identification of each chromosome, were analysed in a few hundred mitoses. ASG- and pancreatin treatment resulted in patterns of dark and lighter bands which are illustrated in Fig. 1. We found good coincidence in staining reaction after the two different procedures.

The ASG-bands of each chromosome were determined by measurement of 10 karyotypes. Number and position of the bands vary dependent on the degree of condensation. Bands, which are discernible in elongated chromosomes, may fuse in highly contracted ones, where fewer, more extended bands occur. The idiogram shown in Fig. 2 illustrates the position of bands as a mean of 10 mitoses. In the idiogram we used two grades of intensity for the bands, because this gives a better representation of the different gradations of staining intensity.

C-bands: Identification of all chromosomes of a complement is possible not only after G-banding, but also after C-banding procedure. Fig. 3 presents the distribution of heterochromatin in the karyotype of *Cricetus cricetus*. Each chromosome shows a large amount of centromeric heterochromatin. Apart from this, there are a few conspicuous bands to be seen. They are situated as follows: one band at the middle of the long arm of chromosome no. 2; two bands at the short arm of chromosome no. 3; two bands near the centromeric region of chromosome no. 4, the band on the long arm appearing more intensive than that on the short arm; one band at the middle of the short arm and one up to three bands on the long arm of no. 5; one band in the distal part of the long arm of no. 6. Chromosome no. 9 varies in its appearance. This may be due to a polymorphism of the chromosome as well as to slight differences in the treatment of several slides. In many cases, chromosome no. 9 is darkly stained throughout its length, in other cases it is only slightly darker than the euchromatic regions of other chromosomes and shows an additional intense band on the long arm. — Both sex chromosomes possess large heterochromatic regions, but we were able to discern three different kinds of staining intensity. The X- and Y-chromosome show a very large centromeric region as intensively stained as the centromeres of the other chromosomes. The short arms of both sex chromosomes are darker stained than the euchromatic zones, whereas the long arm of the Y-chromosome appears not so darkly stained as the centromere, but darker than the short arm. It must be said here that the three gradations are not always distinguishable. Sometimes the Y-chromosome seems to be dark as a whole, but in spite of that, the centromeric region is discernible in most of such cases. There remains to be mentioned a weak heterochromatic band that may be found in the distal part of the euchromatic long arm of the X-chromosome.

Examination of mitoses from different individuals revealed a polymorphism concerning especially the extent of centromeric heterochromatin in the sex chromosomes. In Fig. 4 some types of X- and Y-chromosomes are demonstrated. The varying amount of heterochromatin may even lead to differences in arm ratio. We found X-chromosomes which were metacentric and others which were submetacentric.

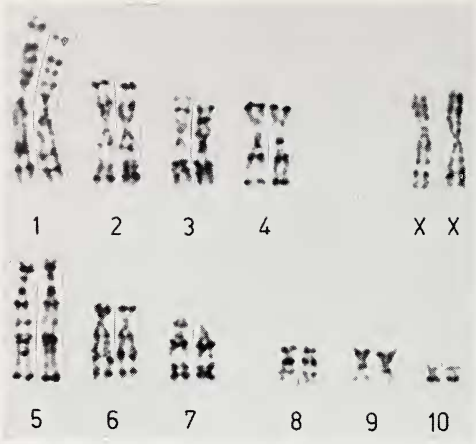

Fig. 1. Female karyotype of *Cricetus cricetus* after ASG-banding procedure (note pair no. 7 as an example for polymorphism in autosomes

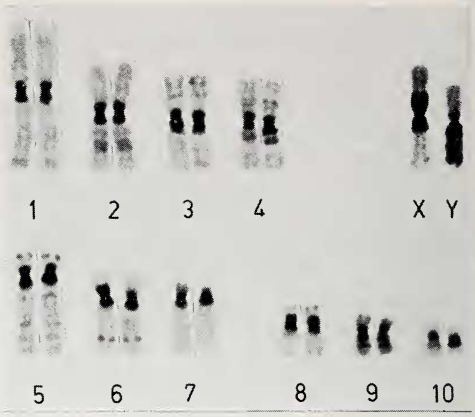

Fig. 3. Distribution of heterochromatin in a male karyotype (note the polymorphic chromosomes in pair no. 7, which correspond to those in Fig. 1)

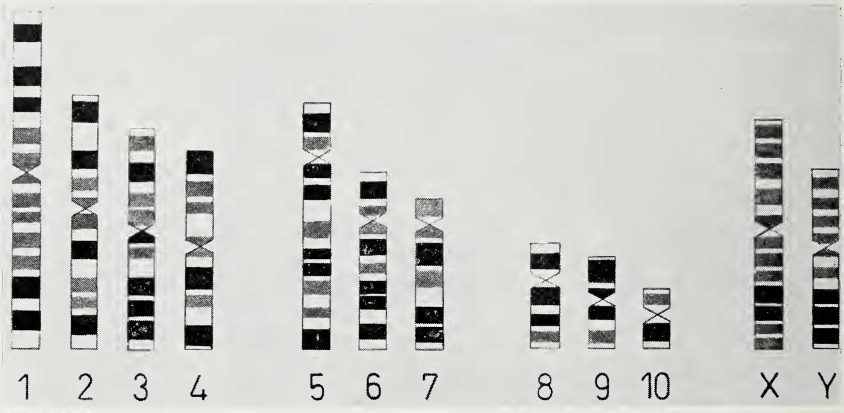

Fig. 2 (above). Schematic representation of G-banding pattern, showing the characteristic bands observed in most metaphases

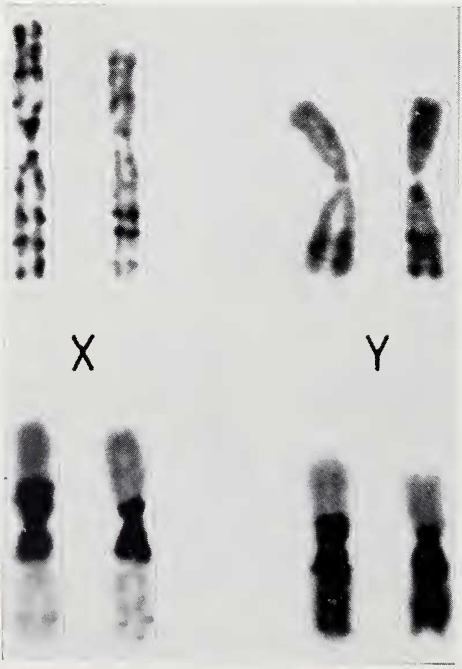

Fig. 4 (left). Polymorphic sex chromosomes (two types of X- and two types of Y-chromosomes; in the first line there are represented G-bands, in the second one C-bands of the corresponding chromosomes)

Biometric study of *Cricetus cricetus* karyotype

Chromosome no.	Relative length		Centromeric index		Arm index	
	mean	standard error	mean	standard error	mean	standard error
1	164,33	±8,78	45,87	±1,21	1,18	±0,06
2	121,60	±6,91	44,93	±1,66	1,23	±0,08
3	106,59	±4,62	46,90	±1,77	1,13	±0,08
4	97,03	±5,70	47,03	±1,27	1,13	±0,06
5	122,31	±6,56	22,88	±1,46	3,39	±0,28
6	88,64	±4,45	26,77	±1,37	2,74	±0,19
7	71,41	±3,96	15,15	±2,45	5,78	±1,20
8	49,52	±5,04	34,32	±2,95	1,94	±0,26
9	46,31	±3,72	46,60	±1,77	1,15	±0,08
10	26,57	±4,08	45,92	±2,68	1,18	±0,13
X	107,29	±5,90	45,16	±2,63	1,22	±0,14
Y	82,40	±6,93	44,34	±2,13	1,26	±0,12

In female individuals, we observed two different situations: either two equal X-chromosomes or two different ones were present. — The polymorphism affected not only sex chromosomes, but also autosomes (see for example chromosome no. 7 in Figs. 1 and 3). In any case, polymorphism was due to supplementary amounts of heterochromatin (Fig. 4).

II. Meiotic chromosomes

Metaphases I and II of male meiosis were analysed by orcein and C-staining. After orcein staining, only a few bivalents can be classified according to the mitotic chromosomes, but application of C-banding procedure, which results in darkly stained centromeric regions especially in the autosomes, makes it possible to identify

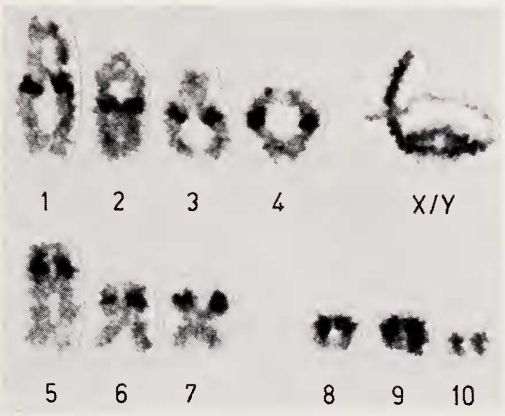

Fig. 5. Male meiosis: Metaphase-I-bivalents after C-staining procedure

each bivalent with sufficient certainty (Fig. 5). Also metaphase-II-chromosomes can be easily identified after C-banding (Fig. 6a, b).

In 50 orcein stained metaphases I, we counted the number of chiasmata. The lowest number in one cell was 17, the highest 22. The average number of 19,36 chiasmata per cell means a chiasma frequency of 1,76 per chromosome. The smaller

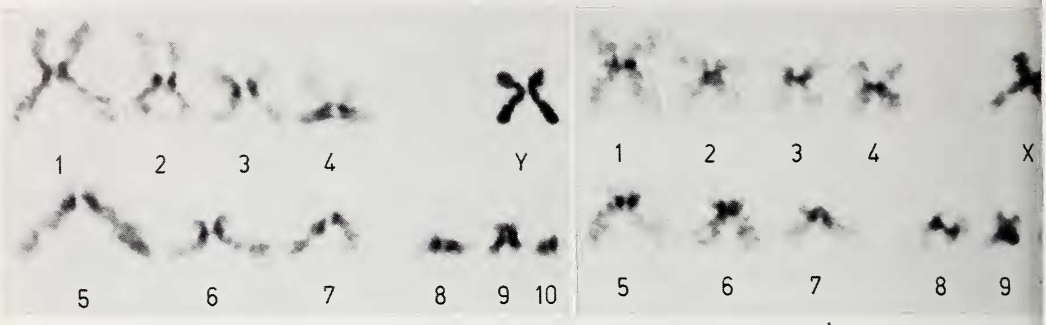

Fig. 6. C-banded metaphase II of male meiosis. a: with X-chromosome; b: with Y-chromosome

chromosomes build up only one chiasma and form rod or cross like bivalents. Longer chromosomes form ring like bivalents with two or three chiasmata. As in other hamster species *(Cricetulus griseus, Cricetulus migratorius, Phodopus sungorus),* the easily recognizable XY-bivalent exhibits a visible interstitial chiasma. The sex chromosomes which both have a slightly stained heterochromatic short arm, appear to build up a chiasma with these short arms. In metaphase II, the entire Y-chromosome is stained strongly, whereas the X-chromosome shows a darker colouring only of the short arm. In addition to this, chromosome no. 9 appears to be darker stained troughout its length, corresponding to the C-staining in mitosis. The weak interstitial bands of heterochromatin, which can be seen in mitotic chromosomes, do not appear in the meiotic ones. This may be due to the diffuse fuzzy structure of meiotic chromosomes.

Discussion

Cytogenetical studies of *Cricetus cricetus* have been carried out previously (Matthey 1952, Fredga and Santesson 1964, Wolf and Hepp 1966, Schmid 1967, Hsu and Benirschke 1970), but the identification of chromosomes — based on their length and structural characteristics — was difficult, especially concerning the chromosomes nos. 2, 3, 4 and the sex chromosomes. Only with use of G- and C-banding, identification is much easier, because each chromosome has its characteristic distribution of bands and heterochromatin. The banding pattern was also examined after staining with quinacrine mustard (method of Caspersson et al. 1970), but we did not observe any striking difference to G-bands. No brightly fluorescent bands can be found.

In the genome of *Cricetus cricetus,* the amount of centromeric heterochromatin is considerably increased in comparison to that of *Cricetulus griseus* and *Phodopus sungorus* (Hsu and Arrighi 1971, Kakati and Sinha 1972). The last mentioned species are interesting because of their lack of centromeric heterochromatin in some autosomes, which is a remarkable contrast to *Cricetus cricetus.* A detailed comparison of G- and C-banding patterns of *Cricetus cricetus* and *Cricetulus griseus* will be dealt with in a separate paper which is in preparation. While *Mesocricetus newtoni* and *Mesocricetus auratus* (Voiculescu et al. 1972, Voiculescu 1974) have entire heterochromatic arms in several autosomes, *Cricetus cricetus* shows this in sex chromosomes only. These sex chromosomes reveal considerable differences to those of other hamster species, especially when the heterochromatic block in the centromeric region is concerned, which is as distinct as in the autosomes. We do not know any hamster with a Y-chromosome comparable to that of *Cricetus cricetus.* Neither the well

defined centromeric area nor the three degrees of staining intensity can be found in others.

As to the X-chromosome, we were able to discern a similarity to other hamsters, such as *Cricetulus griseus* (Kato and Yosida 1972), *Cricetulus barabensis* (Radjabli and Kriukova 1973), *Phodopus sungorus* (Thust 1974), *Mesocricetus newtoni* and *Mesocricetus auratus* (Voiculescu 1974): all of them have two characteristic G-bands localized on the euchromatic arm.

We suppose that the different staining intensities after C-banding procedure are an indication for different categories of heterochromatin (Comings 1972, Jalal et al. 1974), but at the present state of our investigations, we cannot analyse the molecular composition of heterochromatin. On the other hand, comparison of late DNA replication pattern with C-bands is possible to a limited degree only, because previous authors (Pathak et al. 1973, Pathak et al. 1973) have found out that there is no strict correlation between type of heterochromatin and phase of DNA replication.

With regard to our results in meiotic preparations, there is the following observation that demands interest: the chiasma of the XY-bivalent. This chiasma points to homologous segments in the short arms of both sex chromosomes. Here the question may be posed, which category of heterochromatin is present, and wether this DNA material is genetically active or inactive.

In the literature, there are no data available concerning the distribution of heterochromatin in meiotic chromosomes of other hamster species, so that we are not able to compare our results with those of other authors. There are only our own first investigations of *Phodopus sungorus*, which yielded the same results: In both hamster species the distribution of heterochromatin in meiotic chromosomes is in conformity with that in mitotic chromosomes. We intend to complete our investigations of meiosis in *Phodopus* and other hamster species.

Summary

In this study chromosomes of the European hamster (*Cricetus cricetus*, 2 n = 22) are described. Mitotic and meiotic preparations were stained by several different techniques. The chromosomes can easily be identified by G- and C-banding pattern. In the karyotype of *Cricetus cricetus*, there can be found a large amount of centromeric heterochromatin as well as additional heterochromatic bands. Sex chromosomes reveal three different staining intensities after C-banding procedure. — Polymorphism in sex chromosomes and autosomes was observed.

Resumé

Analyse des chromosomes mitotiques et meiotiques du Hamster européen, Cricetus cricetus (L.)

Cette étude présente une description des chromosomes du hamster européen (*Cricetus cricetus*, 2 n = 22). Des préparations de mitoses et de meioses étaient colorées utilisant des méthodes différentes. La disposition des bandes G et C permet d'identifier facilement chaque chromosome. Le caryotype de *Cricetus cricetus* révèle une grande quantité d'hétérochromatine centromerique et aussi des bandes hétérochromatiques supplémentaires. Après l'application de la technique des bandes C, on peut distinguer trois intensités de coloration dans les chromosomes sexuels. — Un polymorphisme des chromosomes sexuels et de quelques autosomes a été observé.

Zusammenfassung

Untersuchungen an Mitose- und Meiosechromosomen des Europäischen Hamsters, Cricetus cricetus (L.)

In der vorliegenden Arbeit werden die Chromosomen des europäischen Hamsters (*Cricetus cricetus*, 2 n = 22) beschrieben, wie sie sich nach Anwendung mehrerer verschiedener Färbemethoden in Mitose und Meiose darstellen lassen. Anhand ihres charakteristischen G- und C-Banden-Musters können alle Chromosomen leicht identifiziert werden. Die C-Färbung

verdeutlicht eine Anzahl bemerkenswerter Kennzeichen des Karyotyps von *Cricetus cricetus:* neben interkalaren heterochromatischen Bändern fallen vor allen Dingen hohe Anteile an Heterochromatin in den Zentromerenregionen auf. An den Geschlechtschromosomen können drei Färbungsintensitäten unterschieden werden, die als Hinweis auf verschiedene Arten von Heterochromatin angesehen werden. — Sowohl an Geschlechtschromosomen als auch an einigen Autosomen wurde ein Polymorphismus beobachtet.

References

Arrighi, F. E.; Hsu, T. D. (1971): Localization of heterochromatin in human chromosomes. Cytogenetics **10**, 81—86.

Caspersson, T.; Zech, L.; Johansson, C.; Modest, E. J. (1970): Identification of human chromosomes by DNA-binding fluorescent agents. Chromosoma (Berl.) **30**, 215—227.

Comings, D. E. (1972): The structure and function of chromatin. Advanc. Hum. Genet. **3**, 237—431.

Evans, E. P.; Breckon, G.; Ford, C. E. (1964): An air-drying method for meiotic preparations from mammalian testes. Cytogenetics **3**, 289—294.

Fredga, K.; Santesson, B. (1964): Male meiosis in the Syrian, Chinese and European hamsters. Hereditas (Lund) **52**, 36—48.

Hsu, T. C.; Arrighi, F. E. (1971): Distribution of constitutive heterochromatin in mammalian chromosomes. Chromosoma (Berl.) **34**, 243—253.

Hsu, T. C.; Benirschke, K. (1970): An atlas of mammalian chromosomes. Vol. **4**, New York: Springer.

Jalal, S. M.; Clark, R. W.; Hsu, T. C.; Pathak, S. (1974): Cytological differentiation of constitutive heterochromatin. Chromosoma (Berl.) **48**, 391—403.

Kakati, S.; Sinha, A. K. (1972): Banding patterns of Chinese hamster chromosomes. Genetics **72**, 357—362.

Kato, H.; Yosida, T. H. (1972): Banding patterns of Chinese hamster chromosomes revealed by new techniques. Chromosoma (Berl.) **36**, 272—280.

Matthey, R. (1952): Chromosomes des Muridae (Microtinae et Cricetinae). Chromosoma (Berl.) **5**, 113—138.

Müller, W.; Rosenkranz, W. (1972): Rapid banding technique for human and mammalian chromosomes. Lancet **1**, 898.

Pathak, S.; Hsu, T. C.; Arrighi, F. E. (1973): Chromosomes of *Peromyscus* (Rodentia: Cricetidae). IV. The role of heterochromatin in karyotypic evolution. Cytogenet. Cell Genet. **12**, 315—326.

Pathak, S.; Hsu, T. C.; Utakoij, T. (1973): Relationships between patterns of chromosome banding and DNA synthetic sequences: A study on the chromosomes of the Seba's fruit bat, *Carollia perspicillata*. Cytogenet. Cell Genet. **12**, 157—164.

Radjabli, S. I.; Kriukova, E. P. (1973): A comparison of banding patterns in two hamster species: *Cricetulus barabensis* and *Cricetulus griseus*. Tsitologiya **15**, 1527—1531.

Rosenkranz, W. (1973): Quantitative Analyse chromosomaler Bandenmuster. Tagung d. Ges. f. Anthrop. u. Humangenet., Gießen, 10.—13. Oktober.

Schmid, W. (1967): Heterochromatin in mammals. Arch. Klaus-Stift. Vererb.-Forsch. **42**, 1—60.

Schnedl, W. (1971): Analysis of the human karyotype using a reassociation technique. Chromosoma (Berl.) **34**, 448—454.

Seabright, M. (1972): The use of proteolytic enzymes for the mapping of structural rearrangements in the chromosomes of man. Chromosoma (Berl.) **36**, 204—210.

Sumner, A. T. (1972): A simple technique for demonstrating centromeric heterochromatin. Exp. Cell. Res. **75**, 304—306.

Sumner, A. T.; Evans, H. J.; Buckland, R. (1971): New technique for distinguishing between human chromosomes. Nature (Lond.) New Biol. **232**, 31—32.

Thust, R. (1974): G-banding and late replication of the Djungarian dwarf hamster chromosomes. Exp. Path. **9**, 153—156.

Voiculescu, I. (1974): A comparative study of the chromosome banding patterns of *Mesocricetus newtoni* and *Mesocricetus auratus*. Z. Säugetierkunde **39**, 211—219.

Voiculescu, I.; Vogel, W.; Wolf, U. (1972): Karyotyp und Heterochromatinmuster des rumänischen Hamsters *(Mesocricetus newtoni)*. Chromosoma (Berl.) **39**, 215—224.

Wolf, U.; Hepp, D. (1966): DNS-Reduplikationsmuster der somatischen Chromosomen von *Cricetus cricetus* (L.). Chromosoma (Berl.) **18**, 438—448.

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